

CYTOGENETIC STUDIES IN THE GENUS *ORNITHOGALUM* L.

III. MEIOTIC ANALYSIS OF SOME INTERSPECIFIC HYBRIDS OF *O. CONICUM* JACQ., AND *O. LACTEUM* JACQ.¹

T. J. ROOS² and R. DE V. PIENAAR³

ABSTRACT

The meiotic chromosome associations as well as the general course of microsporogenesis were investigated in the hybrids of three interspecific crosses involving *O. conicum* Jacq., *O. thyrsoides* Jacq., *O. lacteum* Jacq. and *O. flavissimum* Jacq. The aim was to collect information regarding the evolutionary relationship between these taxa.

The hybrid *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. thyrsoides* (Bokbaai) was almost completely sterile. On backcrossing, however, a few seeds were set, indicating an incomplete barrier to gene exchange. These two species, therefore, bear an ecospecific relationship to one another. Due to a complete sterility of the hybrids between *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. lacteum* (Darling), and *O. lacteum* (Darling) $\times$ *O. flavissimum* (Villiersdorp) the three parental species concerned must be regarded as *cenospecies*.

INTRODUCTION

Sexual reproduction and the accompanying meiotic mechanism play a fundamental role in the course of organic evolution. According to Darlington (1960) it forms a very stable mechanism by which new variation is created and slowly released. Genetic unbalance results whenever two chromosomes fail to pair and form chiasmata. The meiotic process takes place in a causal sequence that is universally maintained, namely chromosome pairing $\rightarrow$ crossing-over $\rightarrow$ chiasmata $\rightarrow$ segregation and reduction $\rightarrow$ sexual reproduction. Crossing-over is the event on which all the evolutionary processes converge, and out of which all the different forms diverge. Crossing-over of gene sequences, or nucleotide sequences, is the basic property of all systems capable of evolution, and sexual reproduction is the mechanism which, in association with crossing-over, has made the higher organisms possible.

Lewis (1957) pointed out that despite the diversity of factors interacting to produce a multitude of variation patterns, there are certain basic principles

¹ Part of thesis submitted by the senior author in partial fulfilment of the requirements of the degree of M.Sc. in Agriculture at the University of Stellenbosch.

² Fruit and Food Technology Research Institute, Stellenbosch.

³ Dept. of Genetics, S.E. College of Agriculture, Stellenbosch.

which permit a sound taxonomy. The most fundamental of these are the genetic discontinuity which results from evolutionary processes and the maintenance of this genetic integrity of the taxa by barriers to gene exchange. The isolating mechanisms operating in plants and animals are fully discussed by Clausen (1951), Dobzhansky (1951), Stebbins (1950) and others. All the cytogenetic work in the field of experimental taxonomy is therefore aimed at detecting the presence or absence of such barriers to gene exchange and, where appropriate, to determine the degree of gene exchange.

This view permits a better understanding of the species concept, which Dobzhansky (1951) defined as follows: "The species is not a static unit, but a stage in the process of evolutionary divergence. Species are formed when a once actually or potentially interbreeding array of Mendelian populations becomes segregated in two or more reproductively isolated arrays." A new species is therefore created when the evolutionary potential is limited to one single group.

It follows that geographical and ecological races, which are units immediately subordinate to the species level, play a fundamental role in speciation.

The pattern of ecotypic differentiation is not constant for all the species, and ecological isolation can occur in different ways. When once this isolation is effective and sufficiently prolonged, the difference between the major groups become so large that hybrids between them are completely sterile. This usually occurs as a result of changes in the genetic make-up and structure of the chromosomes, resulting in intra-generic differences.

Studies in gene-ecology and biosystematics were initiated by Turesson in 1922, but it is mainly due to the exhaustive contributions of Anderson, Babcock, Cleland, Goodspeed, Gustafsson, Müntzing, Stebbins and others, especially Clausen, Keck and Hiesey since 1934, that this field has become an important branch of biology. The biosystematic categories of prime concern are the ecotype, ecospecies and cenospecies.

When there is no barrier to gene exchange between two separate ecological populations, that is, when the hybrids between them are completely fertile, they are termed ecotypes.

The status of ecospecies is reached when an incomplete barrier to gene exchange exists between the two populations. This partial barrier can either be due to genetic incompatibility or hybrid sterility.

When there is a complete barrier to gene exchange between two populations, they bear a cenospecific relationship towards each other.

The ecospecies generally correspond to the species of classical taxonomy.

From the above considerations it would appear that experimental approaches

in the lower ranks of classification can be of value in elucidating the apparent taxonomic confusion.

Leighton (1944-1945) distinguished 80 different indigenous *Ornithogalum* species in South Africa, but indicated that, due to intra-specific variation, the specific differences are not always clear cut.

In the current study a meiotic analysis was made of some *O. conicum* Jacq. and *O. lacteum* Jacq. hybrids to throw light on the genetic relationship between the taxa, especially because very few karyotypic differences could be detected by Pienaar (1963), and Roos and Pienaar (1966) (cf. fig. 1a-d).

MATERIALS AND METHODS

Reciprocal crosses were made, as shown in Table I, between the following biotypes of four *Ornithogalum* species: *O. conicum* Jacq. (Ex Botanic Gardens, Stellenbosch,) *O. thyrsoides* Jacq. (Bokbaai), *O. lacteum* Jacq. (Darling) and *O. flavissimum* Jacq. (Villiersdorp). The localities where the four species were collected are indicated on the map in the paper by Roos and Pienaar (1966). Flower buds of the three viable F_1 hybrids, viz. *O. conicum* $\times$ *O. thyrsoides*, *O. conicum* $\times$ *O. lacteum*, and *O. lacteum* $\times$ *O. flavissimum*, were fixed and treated according to the method of Pienaar (1955). P.M.C. staining proved very successful in the current material.

RESULTS

The results obtained from the reciprocal crosses between the biotypes of the four named *Ornithogalum* species are summarized in Table 1.

TABLE I
 RESULTS OF INTERSPECIFIC CROSSES BETWEEN SOME *ORNITHOGALUM* SPECIES.

♂ Parent ♀ Parent	<i>O. conicum</i>	<i>O. flavissimum</i>	<i>O. lacteum</i>	<i>O. thyrsoides</i>
<i>O. conicum</i>	+	—	×	×
<i>O. flavissimum</i>	—	+	—	—
<i>O. lacteum</i>	S	<	+	—
<i>O. thyrsoides</i>	S	—	—	+

× = viable hybrids

— = no seed set

S = seed set but no viable hybrids produced

+

(a) The hybrid no. 387, viz. *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. thyrsoides* (Bokbaai) produced only a few pollen grains of normal appearance; most of which were 2 $\times$ (Fig. 1 i).

The data from a comparative study of the pollen of the hybrids with the pollen of the parents are summarized in Table II.

TABLE II
NUMBER OF APPARENTLY VIABLE (CARMINE STAINED) AND INVIABLE POLLEN GRAINS, AND THE DIAMETER OF "VIABLE" POLLEN, MEASURED IN μ .

Plant	Number of pollen grains counted	Number of stained pollen grains	% of "viable" pollen grains	Mean diameter (μ)	Standard deviation
<i>O. conicum</i> (Botanic Gardens, Stellenbosch)	518	501	96.7	63	± 2.5
<i>O. thyrsoides</i> (Bokbaai)	533	512	96.1	55	± 3.5
<i>O. conicum</i> $\times$ <i>thyrsoides</i> (No. 387)	499	53	10.6	78*	± 11.3
<i>O. lacteum</i> (Darling)	520	502	96.5	55	± 3.5
<i>O. conicum</i> $\times$ <i>lacteum</i> (No. 390)	530	16	3.0	61	± 5.8
<i>O. flavissimum</i> (Villiersdorp)	556	546	98.2	45	± 3.5
<i>O. lacteum</i> $\times$ <i>flavissimum</i> (No. 1045)	552	49	8.9	72	± 6.5

* In a subsequent measurement of 178 stained grains it was found that 26.7% pollen grains have a diameter between 60–70 μ , 10.5% a diameter between 70–80 μ , 14% a diameter between 80–90 μ , 44.2% a diameter between 90–100 μ , and 4.6% a diameter over 100 μ .

From Table II it is evident that hybrid no. 387 produced only about 11% stained, apparently viable pollen grains. About 66.3% of the hybrid's "viable" pollen grains was approximately twice the volume of the parents' (Table II foot note, and Fig. 1 *h-j*.), and since it is known that polyploidy induces a direct increase in cell volume, it was assumed that this proportion of the hybrid's "viable" pollen grains was diploid. Since the volume of the remaining 33.7% of the "viable" pollen grains approximately equalled that of the parents', they were considered to be haploid. The pollen of this hybrid, therefore, appeared to be a mixture of diploid and haploid pollen grains; this can explain the rather high standard deviation of 11.3 in the pollen grain measurements listed in Table II.

All the P.M.C.'s which entered meiosis were diploid and no apparent abnormalities were detected during anaphase I. In most of the cells meiosis was quite normal resulting in tetrads of four haploid microspores (Fig. 1 *g*). Except for the delayed onset of the second meiotic division in some of the diads no

meiotic abnormalities were detected which could give rise to the high frequency of diploid pollen grains. A preliminary investigation of pollen grain development has as yet failed to throw light on this phenomenon.

TABLE III
 CHROMOSOME ASSOCIATIONS AND CHIASMA FREQUENCIES AT MI OF THE F_1 HYBRID *O. CONICUM* (BOTANIC GARDENS, STELLENBOSCH) $\times$ *O. THYRSOIDES* (BOKBAAL)

Chromosome associations			Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II	III				
	6		8	10.8	± 0.90	1.8
2	5		7	9.0	± 2.10	1.5
4	4		3	7.3	± 0.50	1.2
Total			18	M=9.6	± 2.0	1.5

The mean number of 9.6 chiasmata per cell is indicative of a high degree of genetic homology between the genomes of the two parental species (Tables III and IV, Fig. 1e and f).

The occurrence of a few univalents, as well as the partial reduction in the chiasma frequency compared to that found in natural *O. thyrsoïdes* (Table IV), indicate, however, that some genetic divergence has occurred.

TABLE IV
 CHROMOSOME ASSOCIATIONS AND CHIASMA FREQUENCIES AT MI OF *O. THYRSOIDES* FROM SANDVELD

Chromosome associations			Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasma'a per chromosome pair
I	II	III				
	6		16	12.9	± 1.22	2.2
2	5		4	12.3	± 0.47	2.1
Total			20	M=12.7	± 1.19	2.10

(b) Hybrid no. 390, which resulted from the cross *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. lacteum* (Darling) (cf. Pienaar, 1963) was almost completely sterile. Data concerning the size and viability of pollen grains are summarized in Table II.

The few "viable" pollen grains that were produced appeared normal and haploid and were about the same size as those of the parents (Fig. 2h).

No abnormalities could be detected prior to diakinesis and the pairing of chromosomes was almost complete (Fig. 2a and b). A few univalents and only one quadrivalent were observed in the material. A detailed analysis of meta-phase I cells is given in Table V.

TABLE V
CHROMOSOME ASSOCIATIONS AND CHIASMA FREQUENCIES AT MI OF THE F_1 HYBRID *O. CONI-
CUM* (EX BOTANIC GARDENS, STELLENBOSCH) $\times$ *O. LACTEUM* (DARLING)

Chromosome associations				Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II	III	IV				
	6			31	14.5	± 0.6	2.4
2	5			9	12.7	± 0.6	2.1
1	4	1		1	12.0	—	2.0
	4		1	1	12.0	—	2.0
4	4			5	10.6	± 1.2	1.8
2	3		1	1	12.0	—	2.0
6	3			2	6.0	± 0.0	1.0
Total				50	M=11.4	± 0.6	1.9

Anaphase I was also normal, although a few cells were noted with lagging chromosomes. These, however, were very infrequent. All the later stages appeared to be normal, resulting in apparently normal haploid tetrads.

The differentiation of the pollen grains, however, was rather abnormal. The protoplasmic contents of the microspores commenced to contract at an early stage and soon afterwards degenerated. This irregular behaviour was so frequent that almost 97% of the microspores degenerated, leaving only empty shells (Table II and Fig. 2h).

(c) The cross *O. lacteum* (Darling) $\times$ *O. flavissimum* (Villiersdorp) produced F_1 hybrid no. 1045. Measurements of the pollen grains are summarized in Table II; the mean diameter of 72μ for the "viable" pollen grains revealed a diploid state (Fig. 2i). It was noted that about one quarter of the microsporocytes was polyploid before meiosis commenced. The endomitotic divisions giving rise to these polyploid P.M.C.'s could not be detected.

The normal diploid microsporocytes revealed quite a normal synaptic behaviour resulting mostly in bivalents with readily detectable chiasmata at diakinesis and M.I. (Fig. 2c and d). In the polyploid cells, however, univalents as well as multivalents were formed, which eventually led to an unbalanced segregation of the chromosomes. An analysis of chiasmata in these polyploid cells were very difficult, due to the overlapping and clumping of the chromosomes, but in a number of cells their tetraploid nature was quite evident (Fig.

2e and f). In some instances, however, microsporocytes were noted that had 12 bivalents, (Fig. 2e) and they probably would have completed a normal meiosis resulting in the production of diploid pollen grains.

Table VI summarizes the meiotic analysis of this hybrid.

TABLE VI
 CHROMOSOME ASSOCIATIONS AND CHIASMA FREQUENCIES AT MI OF THE F_1 HYBRID OF *O. LACTEUM* (DARLING) $\times$ *O. FLAVISSUM* (VILLIERSDORP)

Chromosome associations				Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II	III	IV				
(a) <i>Tetraploid</i>							
	12			8	23.7	± 0.54	1.9
2	11			1	22.0	—	1.9
	10		1	3	22.7	± 0.47	1.9
Total				12	M=22.8	± 0.51	1.9
(b) <i>Diploid</i>							
	6			29	12.2	± 0.85	2.0
2	5			5	9.6	± 0.48	1.6
4	4			4	9.0	± 1.00	1.5
Total				38	M=10.6	± 0.71	1.8

The post-meiotic development of pollen grains was similar to that of the other hybrids described. About 91% of the microspores degenerated, leaving 9% surviving pollen grains, all of which were diploid (Fig. 2i).

DISCUSSION

Sympatric, sexually reproducing populations can only be kept intact when gene exchange is limited or prevented by intrinsic genetically conditioned properties, i.e. reproductive isolation. The isolating mechanism can operate in different ways and in the current *Ornithogalum* material it appears that hybrid sterility plays a major part in isolating the different taxa.

According to Dobzhansky (1951) hybrid sterility is due to a specific incompatibility of the parental gene complexes active during meiosis and in post-meiotic development of gametes. He also distinguishes between genic sterility and chromosomal sterility. In the former instance all the meiotic abnormalities such as poor spindle formation and irregular asynapsis and desynapsis are controlled by the genes. Chromosomal sterility is a consequence of an absence or

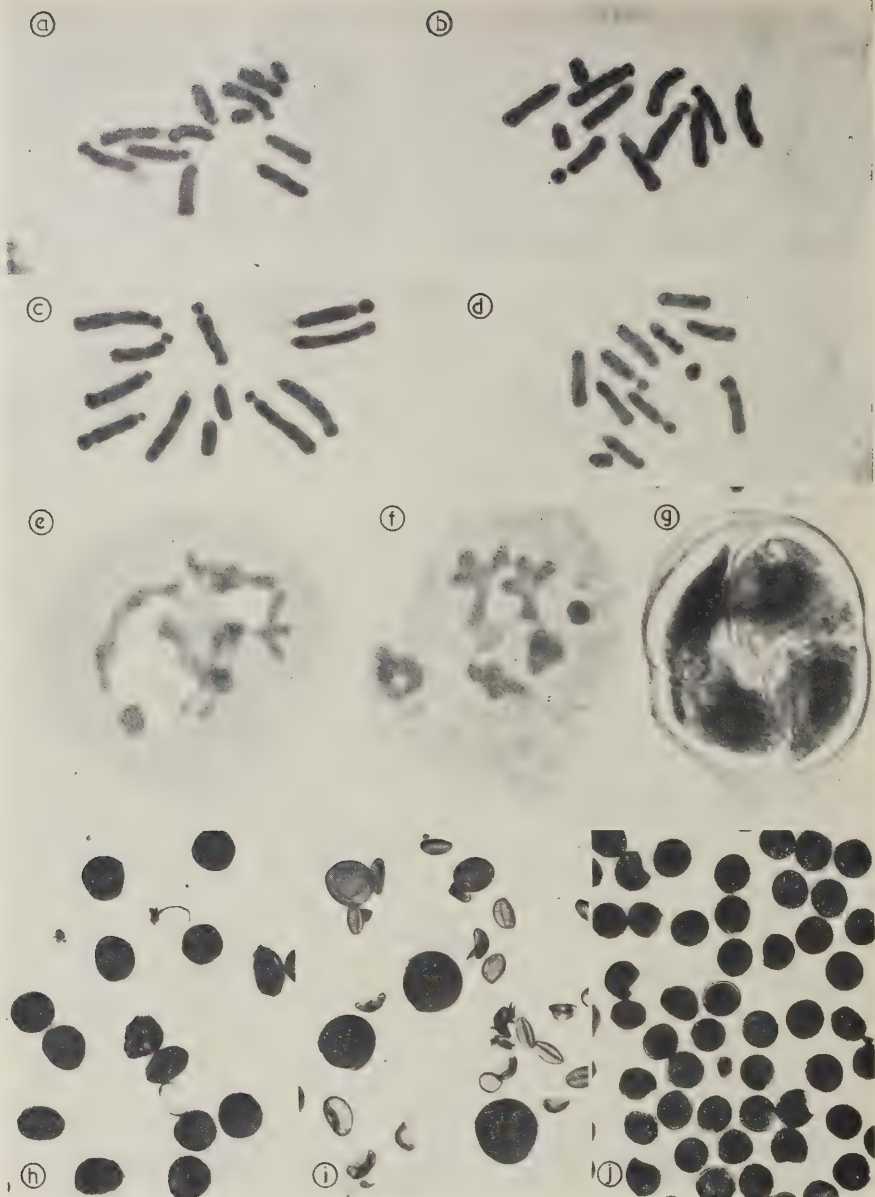


FIG. 1. (a-d) C-mitoses in root tip cells of *Ornithogalum*, $\times 2000$: (a) *O. conicum* (Clanwilliam); (b) *O. thyrsoides* (Bokbaai); (c) *O. lacteum* (Darling); and (d) *O. flavissimum* (Villiersdorp). (e-g). Meiotic stages in hybrid no. 387, viz., *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. thyrsoides* (Bokbaai); (e) and (f) diakinesis, $\times 1500$; (g) Mature tetrad, $\times 1500$. (h-j) Mature pollen grains stained with glycerine-acetocarmine, $\times 250$: (h) *O. conicum* (Ex Botanic Gardens, Stellenbosch); (i) the hybrid no. 387, viz., *O. conicum* (Ex Botanic Gardens Stellenbosch) $\times$ *O. thyrsoides* (Bokbaai); and (j) *O. thyrsoides* (Bokbaai).

reduction of chromosomal homology, that is a mechanical incapacity of normal synapsis.

Stebbins, Valencia and Valencia (1946), however, are of the opinion that no apparent distinction between genic and chromosomal sterility is possible. Greenleaf (1941) showed that in the hybrid *Nicotiana sylvestris* $\times$ *N. tomentosiformes* both these mechanisms work together in producing hybrid sterility.

Nielson (1962) believes that another very important mechanism which regulates fertility, is the balance between the nucleus and the surrounding cytoplasm. Foreign chromatin in the mother cytoplasm results in a disturbance of the normal physiological processes and gives rise, therefore, to sterility.

Because of the fairly normal course of meiosis in the three *Ornithogalum* hybrids described (*O. conicum* $\times$ *O. thyrsoides*, *O. conicum* $\times$ *O. lacteum* and *O. lacteum* $\times$ *O. flavissimum*), independent segregation of the chromosomes of the parental species leads to the formation of haploid spores having various combinations of parental chromosomes; such combinations constitute unbalanced genomes which cannot operate normally in the cytoplasm contributed by the female parental species, and the differentiating spores consequently abort. The greater the genomic differences between the parental species, or the structural hybridity, the greater will be the genic and/or nuclear cytoplasmic unbalance in the spores, with a consequent increase in sterility. In the extreme case only those spores receiving a full normal haploid genome of especially the female parental species (which is a very unlikely event), or an unreduced diploid chromosome complement made up of the genomes of both species, can be expected to function. The rare stainable grains in the hybrid *O. conicum* $\times$ *O. lacteum* may have resulted from the former event, whereas the large apparently functional diploid pollen grains produced by some *Ornithogalum* hybrids (*O. lacteum* $\times$ *O. flavissimum*) can be accounted for on the latter grounds.

The genetic divergence between *O. conicum* (Ex Botanic Gardens, Stellenbosch) and *O. thyrsoides* (Bokbaai) has not continued far enough to cause complete sterility. Although these two species are capable of being crossed, and back crossed with a very limited amount of seed set, chromosomal and genic sterility prevent the free exchange of genes to a large extent. With respect to each other they, therefore, constitute two ecospecies.

The hybrid *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. lacteum* (Darling) revealed an apparently normal meiosis. Despite this, the pollen is almost completely inviable, indicating a high degree of nuclear-cytoplasmic unbalance, or genic relative to chromosomal sterility. Gene differences and/or differences in small fragments of the chromosomes, referred to by Stebbins

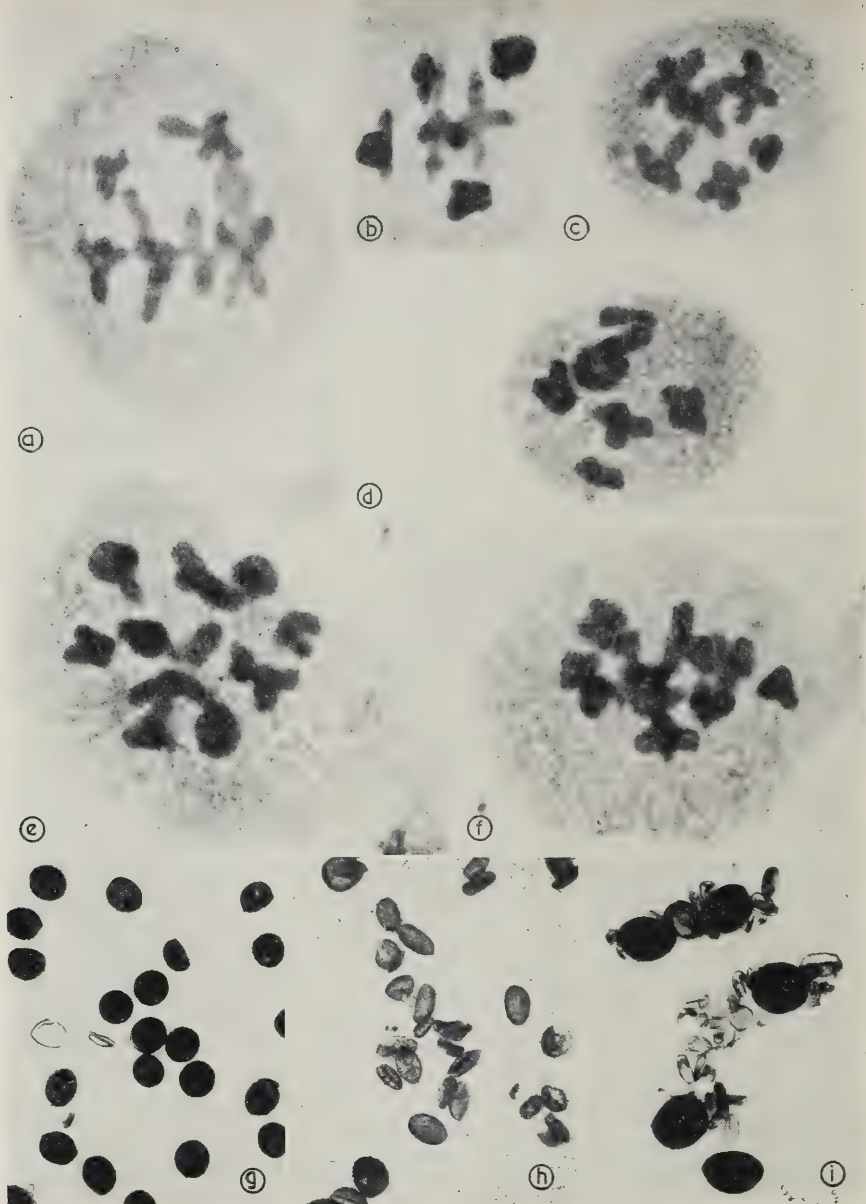


FIG. 2 (a and b) Meiotic stages in hybrid no. 390, viz., *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. lacteum* (Darling): (a) Diakinesis, $\times$ 1500; (b) Metaphase I, $\times$ 1500. (c-f) Meiotic stages in hybrid no. 1045, viz., *O. lacteum* (Darling) $\times$ *O. flavissimum* (Villiersdorp): (c) and (d) Early Metaphase I in diploid P.M.C.'s, $\times$ 1500; (e) and (f) Metaphase I in tetraploid P.M.C.'s, $\times$ 1500. (g-i) Glycerine-acetocarmine stained mature pollen grains, $\times$ 250; (g) *O. lacteum* (Darling); (h) hybrid no. 390, viz., *O. conicum* (Ex Botanic Gardens, Stellenbosch) $\times$ *O. lacteum* (Darling); and (i) hybrid no. 1045, viz., *O. lacteum* (Darling) $\times$ *O. flavissimum* (Villiersdorp).

(1945) as "cryptic structural hybridity", leading to the formation of genetically unbalanced, inviable spores, could basically be responsible for the observed sterility. Such presumed unbalanced gene complexes at the haploid level on the one hand, and the interaction between the foreign chromatin and the maternal cytoplasm (Nielson, 1962) on the other, reduces the viability of the pollen grains to such an extent that no gene exchange is possible between these two species. Consequently, with respect to each other, *O. conicum* and *O. lacteum* must be regarded as two good cenospecies.

Similarly, due to the incapacity of gene exchange between *O. lacteum* (Darling) and *O. flavissimum* (Villiersdorp), and, as shown in Table I, between *O. lacteum* (Darling) and *O. thyrsoides* (Bokbaai), between *O. conicum* and *O. flavissimum* (Villiersdorp), and between *O. flavissimum* (Villiersdorp) and *O. thyrsoides* (Bokbaai), they bear a cenospecific relationship towards each other. In short, they are all good species in the classical taxonomic sense.

ACKNOWLEDGEMENTS

The authors are indebted to the Department of Agricultural Technical Services for making this study possible.

REFERENCES

- CLAUSEN, J., 1951. Stages in the Evolution of Plant Species. Hafner Publ. Co., N.Y.
CLAUSEN, J., KECK, D. D. & HIESEY, W. M., 1947. Heredity of geographically and ecologically isolated races. *Amer. Nat. Vol.*, **81**: 114-133.
DARLINGTON, C. D., 1960. Chromosomes and the theory of heredity. *Nature, Vol.* **187**: 892-895.
DOBZHANSKY, T., 1951. Genetics and the Origin of Species. 3rd revised Ed. Columbia Univ. Press, New York.
GREENLEAF, W., 1941. Sterile and fertile amphidiploids. Their possible relation to the origin of *Nicotiana tabacum*. *Genetics*, **26**: 301-324.
LEIGHTON, F. M., 1944. A revision of the South African species of *Ornithogalum* L. Part I. *Journ. S. Afr. Bot.*, **X**: 83-110.
———, 1944. Part II. *Journ. S. Afr. Bot.*, **X**: 111-122.
———, 1945. Part III. *Journ. S. Afr. Bot.*, **XI**: 135-189.
LEWIS, H., 1957. Genetics and cytology in relation to taxonomy. *Taxon.*, **6**(2): 42-46.
NIELSEN, E. L., 1962. Analogies in the nature and occurrence of sterility in plants. *Crop Science, Vol.* **2**: 181-185.
PIENAAR, R. DE V., 1955. Combinations and variations of techniques for improved chromosome studies in the *Gramineae*. *Journ. S. Afr. Bot.*, **XXI**: 1-9.
———, 1963. Sitogenetiese studies in die genus *Ornithogalum* L. I. Inleidende oorsig. *Journ. S. Afr. Bot.*, **XXIX**: 111-130.
ROOS, T. J. & PIENAAR, R. DE V., 1966. Cytogenetic studies in the genus *Ornithogalum* L. II. Karyotype analyses in some biotypes of *O. conicum* Jacq., *O. lacteum* Jacq., and *O. thyrsoides*. *Journ. S. Afr. Bot.*, **32**: 211-227.
STEBBINS, G. L., 1945. The cytological analysis of species hybrids. *Bot. Rev.*, **11**: 463-486.
———, 1950. Variation and Evolution in Plants. Columbia Univ. Press, N.Y.
VALENCIA, J. I. & VALENCIA, R. M., 1946. Artificial and natural hybrids in the *Gramineae*, tribe *Hordeae*. I. *Elymus*, *Sitanion* and *Agropyron*. *Amer. J. Bot.*, **33**: 338-351.
TURESON, G., 1922. The genotypical response of the plant species to the habitat. *Hereditas*, **3**: 341-347.